

Genetic Polymorphism of *CYP2D6* and Lung Cancer Risk¹

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Abstract

Previous reports of the association of extensive debrisoquine metabolism, controlled by the cytochrome P450 *CYP2D6*, with increased lung cancer risk have been conflicting. We examined the hypothesis that genetic polymorphism at the *CYP2D6* locus identifies individuals at increased risk for lung cancer in a case-control study of 98 incident Caucasian lung cancer patients and 110 age-, race-, and sex-matched controls conducted at the National Naval Medical Center, Bethesda, MD. Using germ line DNA, we identified inactivating mutations at the *CYP2D6* locus (*CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6A*), as well as those mutations that impair but do not abolish enzyme activity (*CYP2D6*9* and *CYP2D6*10A*). Compared to subjects with homozygous inactivating mutations, no association with lung cancer was observed for those with homozygous or heterozygous functional alleles (odds ratios were 0.4 and 0.7, respectively). Furthermore, no excess risk was seen in any histological group or smoking category, and adjustment for smoking and sociodemographic characteristics did not alter the findings. Although the concept that genetic polymorphisms may contribute to differential lung cancer susceptibility is sound, these data do not support the role of *CYP2D6* as a marker for elevated lung cancer risk.

Introduction

The differential susceptibility of cigarette smokers to lung cancer has prompted investigation of other contributing etio-

logical factors. Most chemical carcinogens require metabolic activation to their procarcinogen form. Genetic variation in the ability to activate or detoxify chemical carcinogens could have important consequences in individual cancer risk related to the carcinogen exposure. The polymorphism of the cytochrome P450 gene encoding debrisoquine 4-hydroxylase is one of the most widely studied human drug oxidation defects (1-5). Studies indicate that 3-9% of Caucasians are deficient in this enzyme and are considered poor metabolizers (1, 6-12). Human cytochrome P450 *CYP2D6* may be involved in the activation of carcinogens present in cigarette smoke, including 4-(*n*-methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (13). It is further hypothesized that polymorphisms in this locus may result in differing likelihood of smoking (14). Previous studies using debrisoquine metabolic phenotype have reported an association of extensive metabolism of debrisoquine with increased lung cancer risk (15-18), although others have found no association (19-22). These studies are limited by the inability to distinguish homozygous wild-type extensive metabolizers from heterozygous subjects by phenotyping.

The molecular basis for the deficient metabolism of debrisoquine is a mutation in the *CYP2D6* locus on chromosome 22, which results in an absent or dysfunctional cytochrome P450 enzyme (23, 24). Initial molecular approaches were unable to predict the metabolic phenotype (25), necessitating debrisoquine administration for phenotype determination. The sequencing of *CYP2D* (26) and the subsequent identification of the predominant mutations contributing to deficient metabolism have led to the development of PCR techniques that, with RFLP analysis to detect deletion of *CYP2D6*, are believed to account for 90-95% of mutations (25-29). There remains substantial overlap in metabolic ratios between homozygous wild-type and heterozygote genotypes (25, 30). The nomenclature for describing the human *CYP2D6* alleles has recently been standardized (31).

The published lung cancer case-control studies using *CYP2D6* genotyping have had conflicting results (32-34). Our case-control study of debrisoquine metabolic phenotyping and lung cancer risk enrolled frequency matched hospital-based controls and had an 80% power to detect an OR of 2.7 for excess lung cancer risk among extensive metabolizers (35). We now present our findings on lung cancer risk and the *CYP2D6* genotype.

Subjects and Methods

A case-control study of incident lung cancer was conducted at the National Naval Medical Center, Bethesda, MD, from August 1988 through February 1992. The protocol was approved by all participating institutional review boards, and all study subjects gave signed informed consent. Patients undergoing evaluation for possible lung cancer were identified and recruited from all departments that manage lung cancer patients, and phlebotomy and metabolic phenotyping were conducted before any therapy for lung cancer. Cases were included in the analysis only after their histological diagnosis of lung cancer

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was confirmed by pathology review. Control subjects were recruited from outpatients with scheduled appointments in the Urology and Orthopedic Surgery clinics at the National Naval Medical Center in Bethesda, MD, and matched to cases by 5-year age group, sex, and race. Control subjects with a history of any prior malignancy other than excised basal cell carcinoma of the skin and case subjects with any active malignancy within the prior five years or who had already received chemotherapy or radiotherapy for lung cancer were excluded.

Questionnaire and Medical Record Review. An in-person interview requiring approximately 45 min was administered to the subjects by a trained interviewer. Data collected included sociodemographic characteristics, recent and past tobacco use, personal medical history, caffeine and vitamin use within the prior month, alcohol use, family history of cancer and lung disorders, current medications, and lifetime occupation and residence history. Case medical records were reviewed to abstract selected information including histological diagnoses from pathology reports, results of clinical and pathological staging, and medications administered. Control medical records were reviewed for current diagnoses, history of medical illness, and current medications.

Laboratory Methods. Blood samples (20 ml) were collected into heparinized tubes from patients before they received any treatment for their lung cancer and at the time of interview for control subjects. Samples were processed immediately to isolate lymphocytes for immediate extraction of genomic DNA or for transformation of the lymphocytes with EBV and subsequent extraction of DNA from the cell line.

A number of methods have been developed to identify inactivating mutations of *CYP2D6*. Complete deletion of the wild-type allele *CYP2D6*5* was detected by Southern blot analysis using *Xba*I (36). PCR analysis was used to detect the other known alleles: *CYP2D6*3* (deletion of A₂₆₃₇ in exon 5), *CYP2D6*4*, (G₁₉₃₄→A at intron 3/exon 4 splice site), *CYP2D6*9* (deletion of Lys₂₈₁), *CYP2D6*10A* (C₁₈₈→T in exon 1 and G₄₂₆₈→C in exon 9), and *CYP2D6*6A* (deletion of T₁₇₉₅ in exon 3) (26, 27, 37–39). There is no detectable enzyme activity when *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, or *CYP2D6*6A* are present. Decreased enzyme activity is seen with *CYP2D6*9* and *CYP2D6*10A* (31).

Primer-directed mismatch PCR-based tests for the mutations were developed to facilitate screening large numbers of samples (40, 41). For this analysis, alleles considered to be associated with inactive enzyme were *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, or *CYP2D6*6A*. PCR analysis was conducted blinded from phenotyping results. The mismatch PCR-based tests involved the use of two complementary tests: the mutant cuts and wild type cuts tests. Both tests were performed on each sample and had to give confirmatory results.

Statistical Methods. The relationship of genotype to lung cancer was assessed by the OR⁵ approximation of the relative risk, and 95% confidence intervals were obtained from logistic regression models performed with the BMDP statistical analysis program (42). Stratified and multivariate analyses were used to examine the data for confounding and effect modification. Confounding by smoking was assessed in several ways; by status (never, former, or current); a combination variable (never smokers, former smokers of 20 cigarettes per day or less, former smokers of more than 20 cigarettes per day, current

Table 1 Distribution of cases and controls by sociodemographic features and cigarette smoking history

	Controls (n)	Cases (n)
Male	71	66
Female	39	32
Education		
High school	15	33
College	40	38
Graduate School	55	27
Smoking history		
Never	50	5
Former	47	33
≤20 cpd ^a	20	8
>20 cpd	27	25
Current	13	60
≤20 cpd	7	8
>20 cpd	6	52
Mean age (yr)	59.6	58.5
Mean BMI	24.6	24.8

^a cpd, cigarettes per day.

smokers of 20 cigarettes per day or less, or current smokers of more than 20 cigarettes per day); pack-years of smoking in quartiles; and as a continuous variable with smoking status as a covariate. Because *CYP2D6* genotype OR estimates were similar regardless of which smoking variables were used, smoking status was included in the final model. Former smokers were individuals who had not smoked in the year prior to the diagnosis for cases and the year prior to interview for controls. Pack-years were calculated as one-twentieth the product of the total number of years of smoking and the average number of cigarettes smoked per day. ORs presented are adjusted for age (tertiles), sex, education (≤12 years, 13–16 years, or >16 years), and smoking status (never, former, or current).

Results

Ninety-eight of 109 (90%) Caucasian patients with histologically confirmed lung cancer and 110 of 135 (81%) age-, race-, and sex-matched controls had genotyping for *CYP2D6*. Subjects without genotyping results refused phlebotomy.

A majority of subjects were male (66%), with a mean age of 59 years for both cases and controls (Table 1). Patterns of cigarette use were typical for studies of lung cancer; cases smoked much more heavily (on average, cases smoked about 30 cigarettes per day versus 20 cigarettes per day among controls) and for a longer time (36 versus 28 years for cases and controls, respectively), resulting in a mean of 56 pack-years for cases compared to 30 pack-years among smoking controls. Nearly 50% of the controls reported never smoking cigarettes, and most had some years of college education (86%). Mean BMI was the same for both groups.

The distributions of the *CYP2D6* genotype (based on inactive enzyme) among controls and cases are shown in Table 2. Most cases were of adenocarcinoma (35), SCLC (30), or squamous cell carcinoma (16); the remaining group of other histology (17) comprised three large cell carcinoma, eight NSCLC, and six mixed histology. The distribution of *CYP2D6* genotypes did not differ between cases and controls ($P = 0.15$; 54 and 57% of cases and controls, respectively, were homozygous wild-type), and genotypes in each histological group were similar to the case group overall. The allele frequencies did not differ between cases and controls or between case histologies;

⁵ The abbreviations used are: OR, odds ratio; BMI, body mass index; SCLC, small cell lung cancer; NSCLC, non-SCLC.

Table 2 Distribution by *CYP2D6* genotype (based on inactivating alleles) and lung cancer histology

<i>CYP2D6</i> genotype	Controls	Cases				
		All	Squamous cell carcinoma	SCLC	Adenocarcinoma	Other ^a
<i>CYP2D6</i> *1A/ <i>CYP2D6</i> *1A	63	53	9	17	19	8
<i>CYP2D6</i> *1A/ <i>CYP2D6</i> *3	1	1	0	0	1	0
<i>CYP2D6</i> *1A/ <i>CYP2D6</i> *4	36	33	5	10	11	7
<i>CYP2D6</i> *1A/ <i>CYP2D6</i> *5	4	4	1	1	1	1
<i>CYP2D6</i> *1A/ <i>CYP2D6</i> *6A	3	0	0	0	0	0
<i>CYP2D6</i> *3/ <i>CYP2D6</i> *3	0	0	0	0	0	0
<i>CYP2D6</i> *3/ <i>CYP2D6</i> *4	1	0	0	0	0	0
<i>CYP2D6</i> *3/ <i>CYP2D6</i> *6A	0	0	0	0	0	0
<i>CYP2D6</i> *4/ <i>CYP2D6</i> *4	1	7	1	2	3	1
<i>CYP2D6</i> *4/ <i>CYP2D6</i> *6A	1	0	0	0	0	0
Total	110	98	16	30	35	17

^a Includes three large cell, eight NSCLC, and six mixed histology cases.Table 3 Distribution by *CYP2D6* allele frequency according to lung cancer histology

Allele	Controls	Cases				
		All	Squamous cell carcinoma	SCLC	Adenocarcinoma	Other
<i>CYP2D6</i> *1A	0.77	0.73	0.75	0.75	0.73	0.71
<i>CYP2D6</i> *3	0.01	0.01	0.00	0.00	0.01	0.00
<i>CYP2D6</i> *4	0.18	0.24	0.23	0.23	0.24	0.25
<i>CYP2D6</i> *5	0.02	0.02	0.03	0.02	0.01	0.03
<i>CYP2D6</i> *6A	0.02	0.00	0.00	0.00	0.00	0.00

Table 4 Debrisoquine metabolic phenotype distribution of lung cancer cases and controls predicted from *CYP2D6* genotype using inactivating mutations

Predicted phenotype	Controls	Cases	OR ^a	95% confidence interval
Poor	3	7	1.00	
Intermediate	44	38	0.27	(0.05–1.56)
Extensive	63	53	0.26	(0.05–1.48)

^a Adjusted for age, sex, smoking, and education.

the frequency for the wild-type allele was 0.77 for controls and 0.73 for cases overall ($\chi^2 = 3.76$; $P = 0.44$; Table 3). Among the histological subtypes, the wild-type allele frequency ranged from 0.71 to 0.75. Gene frequencies for the controls are in Hardy-Weinberg equilibrium (43).

Using genotype results to predict debrisoquine metabolic phenotype predicted fewer poor metabolizers among controls than cases (3 versus 7, respectively, Table 4). Compared to the poor metabolizers, ORs for extensive and intermediate metabolizers were less than 1.00, but they were not statistically significant. Crude and adjusted ORs did not differ. The distribution of predicted phenotypes among the histologies did not differ from the overall case group; a frequency of 6–9% poor metabolizers was predicted for each histological group (Table 5). Persons homozygous for variant alleles made up less than 3% of the controls.

The phenotype-genotype correlation is similar to that reported by others (25). As shown in Fig. 1, there is good correlation of phenotypic extensive metabolizers with the homozygous wild-type genotype. There is a broad range of log metabolic ratio associated with the heterozygous genotype, and the range for the homozygous deleterious alleles spans the range from the tail of the extensive metabolizer distribution to

Table 5 Debrisoquine metabolic phenotype according to lung cancer histology predicted from *CYP2D6* genotype using inactivating mutations

Predicted phenotype	Controls	Cases			
		Squamous cell carcinoma	SCLC	Adenocarcinoma	Other ^a
Poor	3	1	2	3	1
Intermediate	44	6	11	13	8
Extensive	63	9	17	19	8

^a Includes three large cell, eight NSCLC, and six mixed histology cases.

the higher values. The potential misclassification of the two heterozygotes in the graph could be due to the subsequent identification of other inactivating alleles that were not identified at the time of this analysis. Nevertheless, including them with the poor metabolizers in this analysis would not change the results.

Two additional alleles associated with decreased metabolic activity have been described. *CYP2D6**9 has been suggested to be an independent lung cancer risk factor (33), but we found no such excess among our cases (44). Furthermore, the gene frequency of 5% among our controls was consistent with population estimates described to date. *CYP2D6**10A consists of mutations in exons 1 and 9, without the G₁₉₃₄→A mutation at the intron 3/exon 4 splice site. This allele was detected definitively in 2 controls and 1 case; however, in several instances, the status of *CYP2D6**10A was equivocal because amplification of exon 1 was not possible in subjects with exon 9 mutations (17 controls and 12 cases). No lung cancer association was observed for *CYP2D6**10A, regardless of whether or not the equivocal subjects were included.

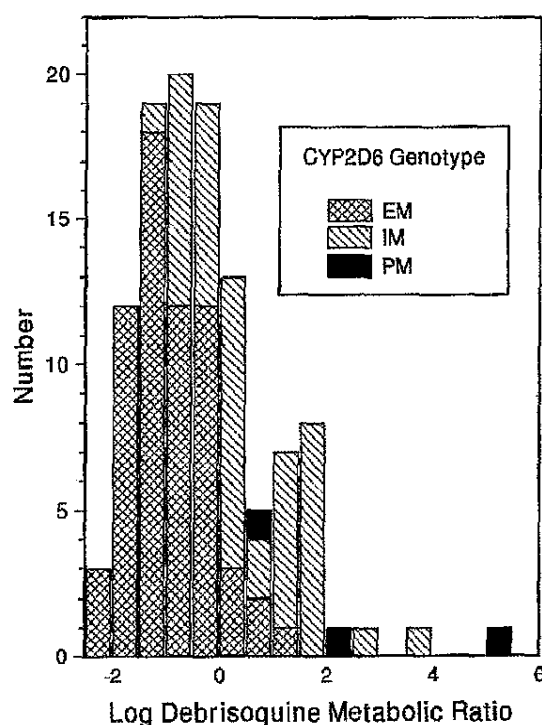


Fig. 1. Distribution frequency of measured natural log debrisoquine metabolic ratio by *CYP2D6* genotype. EM, extensive metabolizers (homozygous wild-type alleles); IM, intermediate metabolizers (heterozygotes); PM, poor metabolizers (homozygous variant alleles).

Additional analyses stratified according to smoking profile, (nonsmoker or light smoker, <20 pack-years; moderate smoker, 20–40 pack-years; and heavy smoker, >40 pack-years) were unremarkable, with an OR for extensive metabolizers less than 1.00 in each instance.

Discussion

We examined 208 Caucasian subjects from a case-control study of lung cancer to determine whether mutations in the *CYP2D6* gene were associated with lung cancer risk as some authors have reported (33, 34), but no case-control differences in the distribution of *CYP2D6* genotypes or variant alleles were found. Our use of an age- and sex-matched case-control study design is unlike most early investigations of lung cancer and *CYP2D6*, in which comparison subjects were recruited without regard to age and controls were much younger than the lung cancer cases (17). Other investigators have not reported smoking history in their controls (33) or matched on smoking and thus could not evaluate smoking as a potential confounder (15–16, 18–19). Although some have argued that matched controls are not necessary to evaluate a genetic trait, it may be that the absence or presence of mutations in the *CYP2D6* gene cluster influences survival. If so, the genotype distribution would differ with age.

It has been hypothesized that because of their metabolic deficiency, poor metabolizers of debrisoquine are less likely to smoke (14); thus, the protective effect for the phenotype ob-

served in some studies may be partly explained by uncontrolled or residual confounding by smoking. We, however, did not observe a notable difference between heavy and light smokers in the lung cancer/debrisoquine genotype association.

The heterogeneity of results among studies examining the debrisoquine metabolic phenotype and/or genotype is not clearly understood (45). Our study had a low proportion of predicted deficient metabolizers. Although certain ethnic groups (i.e., African-Americans and Asians) have a low frequency of mutated alleles (46, 47), this cannot account for the lack of poor metabolizers because our analysis was limited to Caucasians. Our controls tended to be more educated and smoked less than the general population, but it is doubtful that any of these factors contribute to the *CYP2D6* genotype. There has been speculation that certain psychological traits, smoking, various medical illnesses (notably autoimmune disease), and BMI are related to the genotype, but the data in support of each of these are weak at present (48).

Additional variant alleles have been identified since this study was conducted, so some misclassification is possible. However, Marez *et al.* (49) report that five allelic variants account for 87% of all alleles, and Broly *et al.* (29) report that the *CYP2D6*4A* and *CYP2D6*5* allelic variants account for 90% of detrimental mutations. Thus, it is unlikely that misclassification related to the more recently described variant alleles would have significantly altered the results.

A review of previously published studies (50) suggests that the putative risk for the debrisoquine phenotype is limited to the nonadenocarcinoma histologies; the combined OR for extensive metabolizers among adenocarcinomas is 1.0. The proportion of adenocarcinoma in the current study is similar to that in other studies and would not explain our findings. The large number of small cell carcinomas does not appear to be relevant, because our study finds no elevation of risk for any histology. Although the reasons for heterogeneity remain incompletely understood, this study clearly suggests that the *CYP2D6* deficient genotype is not protective for lung cancer.

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